

Influence of Provenance on *Ribes Cereum* and *Symphoricarpos Oreophilus* Seed Germination in New Mexico Seed Sources¹

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Abstract

Mountain snowberry (*Symphoricarpos oreophilus*) and wax currant (*Ribes cereum*) are co-occurring shrub species found in ponderosa pine and mixed conifer forests in New Mexico. These species are candidate species for mined land reclamation because both occur in full sunlight and in the understory and are found on a wide range of edaphic conditions. Mountain snowberry seeds have both a scarification and a stratification requirement for germination, whereas wax currant seeds require only stratification treatment. Separate studies were conducted examining the influence of provenance, from within New Mexico, on conventional seed propagation protocols for each species. The wax currant study utilized eight seed sources and the mountain snowberry study utilized seven seed sources. Seed sources were selected to represent the latitudinal range of the species in New Mexico, and an elevational range at the most northerly latitude sampled. There was considerable variability among seed sources of both species in overall germination rates and response to treatment severity. In wax currant, the southernmost source did not benefit from stratification, but for all of the more northerly sources, germination was improved by stratification treatments. There was also considerable variability among mountain snowberry seed sources in response to scarification treatments, but no distinct latitudinal trends were apparent. Implications of these studies on selection pressure and restoration are discussed.

Additional Key Words: seed dormancy, adaptation, provenance.

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Introduction

Native species are uniquely adapted to local climates and site conditions, and for this reason, they are being intensively studied as potential reclamation species.

Mountain snowberry (*Symphoricarpos oreophilus* Gray) and wax currant (*Ribes cereum* Dougl.) are shrub species native to the Southwest, whose roles in reclamation are being evaluated in New Mexico. These species occur throughout montane regions of western North America at elevations of 1200 to 4000 meters (McMurray 1986, Marshall and Winkler 1995). Mountain snowberry and wax currant are frequently abundant, occur in numerous communities, and inhabit both understory and open microclimates (McMurray 1986, Marshall and Winkler 1995). Mountain snowberry and wax currant co-occur in ponderosa pine and mixed conifer forests in New Mexico. Both species are capable of growing on steep slopes, poor soils, and sites ranging from moist to dry. Mountain snowberry spreads (reproduces) rapidly once established through rhizomes and layering (McMurray 1986) and by seed. In contrast, wax currant reproduces primarily by seed. Both species have colonized disturbed sites at Molycorp Mine in Questa, New Mexico (Harrington- personal observation).

Natural plant invasion and succession occur slowly on most mine sites (Monsen 1984), and planting of nursery-grown native materials can speed up the time scale of revegetation. However, for many native shrub species, propagation techniques are not well researched, resulting in increased production costs (Dreesen and Harrington 1997). In addition, propagation literature is often based on studies involving few seed lots, and fails to take into account ecotypic variability. As a result, recommended protocols may be less than adequate for some seed sources.

Literature on seed propagation techniques for mountain snowberry is sparse. Early works on the seed propagation of common snowberry (*Symphoricarpos albus* Blake) and Indian currant snowberry (*Symphoricarpos orbiculatus* Moench) – two other North American species – have served as models for the genus. Seed dormancy in these species is imposed by both the seed coat and the embryo (Flemion 1934, Pfeiffer 1934, Flemion and Parker 1942).

Seed coat dormancy in *S. albus* is not thought to be due to barriers to water imbibition, but rather, dormancy is attributed to a combination of mechanical resistance of the seed coat and possible physiological control of embryonic tissues exerted by the seed coat (Pfeiffer 1934). Effective seed coat treatments must disintegrate or soften the outer seed coat fibers. Sulfuric acid scarification and moist after-ripening (which enables fungi to infect and soften seed coat fibers) are two techniques that have been used to overcome seed coat dormancy in snowberry species (Flemion 1934, Flemion and Parker 1942, Glazebrook 1941 cited in Krier 1948, Young and Young 1992).

The combination of both acid scarification and moist after-ripening treatments has been found to be most effective in promoting snowberry germination, but optimal acid scarification and moist after-ripening treatment durations vary widely from author to author. Recommended acid soak duration ranges from 20-75 minutes, and recommended treatment duration for subsequent after-ripening ranges from 14 to 120 days (Flemion 1934, Flemion and Parker 1942, Krier 1948, Young and Young 1992).

Snowberry seeds also exhibit embryo dormancy due to embryo immaturity, which is overcome by long periods of stratification (Flemion 1934, Flemion and Parker 1942). Recommendations for stratification treatment duration are less variable than those for scarification – in all cases from four to six months (Flemion 1934, Flemion and Parker 1942, Krier 1948, Evans 1974, Young and Young 1992).

As is the case with mountain snowberry, propagation literature for wax currant is based primarily on studies of closely related species. Several dormancy mechanisms are suspected to occur in some species of *Ribes*. These mechanisms include seed coat dormancy controlled by growth inhibitors and an impermeable seed coat and embryo dormancy resulting from a rudimentary embryo (Pfister 1974, Goodwin and Hummer 1993). However, for wax currant, embryo dormancy is the primary dormancy mechanism, and satisfactory germination has been achieved in the absence of scarification treatments (Pfister 1974). Embryo dormancy of wax currant has been overcome by stratification for a period of 120 to 150 days (Pfister 1974). This treatment resulted in 61% germination. However, when the same seeds underwent a second stratification treatment, an

additional 11% of the original seeds germinated. For other *Ribes* species, there is a high degree of variability in optimal stratification treatment duration. Recommended stratification duration for *R. alpinum*, *R. americanum*, *R. aureum*, *R. cynosbati*, *R. hudsonianum*, *R. inerme*, *R. irriguum*, *R. lacustre*, *R. missouriense*, *R. montigenum*, *R. nevadense*, *R. odoratum*, *R. roezli*, *R. rotundifolium*, *R. sanguineum*, and *R. viscosissimum* range from 60 to 300 days depending on species (Fivaz 1931, Quick 1936, Heit 1971, Pfister 1974, Goodwin and Hummer 1993). Seed dormancy level has been found to vary widely among seed lots (Pfister 1974, Young and Young 1992).

Materials and Methods

Seeds used in both studies were collected during the months of August through October, 1997 at multiple locations (sources) throughout New Mexico (see **Table 1**).

Seeds were collected from a minimum of five plants and varying plant heights at each source. Sources were selected to encompass a range of latitudes within New Mexico and to reflect the range of elevations at the Molycorp Mine in Questa, New Mexico. Identification to species by floral characteristics was not accomplished for snowberry growing at the Sacramento and two Sandia sources. Foliar characteristics, however, were consistent with *S. oreophilus*. While *S. oreophilus* is known to occur at these elevations in these ranges, other species of *Symphoricarpos* may also occur at these locations (Martin and Hutchins 1981).

Following collection, seeds were cleaned and separated into 100-seed lots and placed in dry storage at 5°C until use. The snowberry study consisted of one experiment examining various levels of acid scarification and moist after-ripening treatment. The wax currant study consisted of one experiment examining various levels of stratification duration.

The snowberry study was designed to examine the influence of provenance on germination response to a factorial combination of acid scarification and moist after-ripening treatments. Seven seed sources were used.

Table 1 Lot title, latitude, location, elevation, and collection date of mountain snowberry (*Symphoricarpos oreophilus*) and wax currant (*Ribes cereum*) seed sources.

Lot Title	Latitude	Location	Elevation	Collection Date
Mountain snowberry- <i>Symphoricarpos oreophilus</i>				
Capulin	3642' N	Molycorp Mine, Questa, NM	9,800 ft	9/04/97, 9/24/97
Vent	3642' N	Molycorp Mine, Questa, NM	8,200 ft	9/6/97
Cabin	3642' N	Molycorp Mine, Questa, NM	7,900 ft	9/2/97
Holman	3602' N	Holman, NM	7,800 ft	10/7/97
Sandia Crest	3510' N	Cibola National Forest	9,200 ft	10/11/97
Sandia Trail	3510' N	Cibola National Forest	7,700 ft	10/11/97
Sacramento	3258' N	Cloudcroft, NM	8,600 ft	9/21/97, 10/04/97
Wax currant- <i>Ribes cereum</i>				
Capulin	3642' N	Molycorp Mine, Questa, NM	9,800 ft	8/10/97
Raspberry Ridge	3642' N	Molycorp Mine, Questa, NM	9,800 ft	8/12/97
Pinon Knob	3642' N	Molycorp Mine, Questa, NM	9,500 ft	8/21/97
Headframe Hill	3642' N	Molycorp Mine, Questa, NM	8,400 ft	8/13/97
Mahogany Hill	3642' N	Molycorp Mine, Questa, NM	9,100 ft	8/13/97
Boxcar/Mill	3642' N	Molycorp Mine, Questa, NM	8,200 ft	8/13/97

Table 1 Lot title, latitude, location, elevation, and collection date of mountain snowberry (*Symphoricarpos oreophilus*) and wax currant (*Ribes cereum*) seed sources.

Lot Title	Latitude	Location	Elevation	Collection Date
Rociada	3550' N	Rociada, NM	7,800 ft	8/17/97
Gila	3406' N– 3407' N	Gila National Forest–NM	8,200 ft.	8/21/97

Seeds underwent acid scarification treatment prior to after- ripening treatment. Each of the nine treatment combinations was tested on four 100-seed replications per source. All seeds were then stratified for 168 days. Germination data were analyzed as a three (acid scarification) by three (moist after-ripening) factorial separately by seed source.

Concentrated sulfuric acid (Reagent ACS, 95.0-98.0%, VWR) was used for all acid scarification treatments. Snowberry seeds were exposed to acid for 0, 30, or 60 minutes. Seeds were placed in 10-ml of acid and stirred vigorously for 30 seconds to disperse the seeds. Following treatment the seeds were removed from the acid and rinsed with water for one minute under a running tap.

After-ripening treatment involved mixing snowberry seeds with moistened peat moss, placing the seed/peat mixture into polybags, and storing the polybags at room temperature (21°C to 24°C). The peat moss had been fully saturated and then firmly pressed to remove excess water. Seeds were after-ripened for 0, 21, or 42 days.

Stratification was accomplished by mixing snowberry seeds with moistened peat moss and the seed/peat mixture was placed in polybags stored in a walk-in cooler. Snowberry seeds were stratified for 168 days. Cooler temperatures fluctuated from an average daily low of -1.2°C to an average daily high of 5.4°C.

Following stratification, germinated seeds were counted and removed. Seeds were considered germinated if the radical had emerged through the seed coat. Ungerminated seeds were then incubated to test for germination. Snowberry seeds were tested for germination between filter papers (Whatman 15.0 cm grade #1 qualitative) moistened with distilled water, which were set in 150 ml petri dishes sealed in 15x16 cm polybags. Petri dishes were set 30 cm beneath two 40-watt Sylvania Grow Lux fluorescent bulbs on FloraCart plant stands (Grower’s Supply Company, Dexter, MI). The light cycle was 10 hours of light followed by a 14-hour dark period. Lab temperatures ranged from mean daily highs of 23.4°C +/- .1°C to mean daily lows of 21.7°C +/- .1°C.

The wax currant study evaluated the influence of provenance on germination response to stratification imposed as the only seed treatment. Experimental factors were seed source and stratification duration. Seeds from all eight sources were used. Stratification treatment durations were 0, 60, 90, and 120 days. All treatment combinations were tested with four replications of 100 seeds. Germination data were analyzed as a four (stratification) by eight (seed source) factorial, and then separately by source.

Wax currant seeds were stratified between filter papers (VWR 9.0 cm Qualitative Grade #3) moistened with distilled water, which were placed in 100 mm petri dishes sealed in 15x16 cm self-sealing polybags within a walk-in cooler. Wax currant seeds were stratified for 0, 60, 90, or 120 days. Cooler temperatures again fluctuated from an average daily low of -1.2°C to an average daily high of 5.4°C.

Following stratification, germinated seeds were counted and removed. Seeds were considered germinated if the radical had emerged through the seed coat. Ungerminated seeds were then incubated to test for germination. Wax currant seeds were tested for germination between filter papers (VWR 9.0 cm Qualitative Grade #3) moistened with distilled water, which were placed in 100 mm petri dishes sealed in 15x16 cm self-sealing polybags. Petri dishes in polybags were placed directly on greenhouse benches under natural light (filtered through shade cloth) with fluctuating temperatures. A one-foot border on all sides of each bench was left empty in order to minimize temperature differences between samples. Greenhouse temperatures ranged from a mean daily high of 34.1°C +/- 0.5°C to a mean daily low of 15.2°C +/- .26°C. After 7, 14, 21, and 28 days of incubation, germinated seeds were again counted and removed. Filter papers were remoistened as needed.

Categorical analysis of variance (SAS Proc Catmod, SAS Institute 1989) was used to determine treatment differences using the factorial treatment structures described for each experiment. The response variable was total germination, including both germination during treatment imposition and germination within 28 days after treatment imposition. This procedure is a generalization of the chi-square (X^2) test of homogeneity, which uses the “logit” – the natural log of the ratio of germinated to non-germinated seeds for each treatment – as the response. Maximum-likelihood analysis was used to calculate X^2 test statistics. Observed significance levels less than $\alpha=0.05$ were considered significant. Percentages and standard errors were calculated for main effects and interaction combinations. Approximate pairwise Z statistics were used to conduct pairwise comparisons of main treatment effects using a conservative alpha value of 0.05 divided by the number of comparisons. Pairwise comparisons of treatment combination were informally tested; means were considered different if the higher mean minus its standard error did not overlap the lower mean plus its standard error.

Results

Acid scarification, after-ripening, and for all but two mountain snowberry seed sources (Sandia Trail and Sacramento), the interaction between both factors impacted germination ($p<0.05$). Germination for individual seed sources was low and variable, when averaged over all treatments, and ranged from 8.5% to just over 35.1% (see Table 2).

Table 2 Mountain snowberry germination by seed source for data averaged over all other treatments

Seed Source	Mean Germination Percentage	Standard Error
Capulin	20.9	0.7
Vent	24.4	0.7
Cabin	20.9	0.7
Holman	35.1	0.8
Sandia Crest	31.4	0.8
Sandia Trail	22.1	0.7
Sacramento	8.5	0.5

Acid scarification treatment (when averaged over all levels of after-ripening) improved germination for all snowberry seed sources (see Figure 1 (A)). Thirty minutes was the optimal soak duration for all seed sources except Vent, for which a 60-minute soak was equally effective. Improvement in germination relative to non-acid-scarified seeds ranged from 19% to over 300%, depending on seed source. The response to longer acid scarification treatment was more variable and five of the seven seed sources (all except Holman and Vent) showed no improvement in germination relative to non-scarified seeds. Seeds from Sandia Crest, Sandia Trail, and Sacramento – the three southernmost sources – benefited the least from acid scarification.

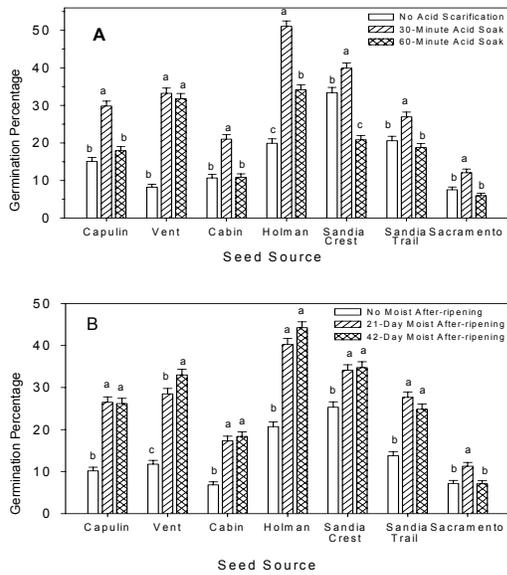


Figure 1. Main Effect of A. Acid scarification and B. Moist after-ripening on mountain snowberry germination.

similar following both after-ripening treatments. Only the Vent seed source had a higher germination rate when exposed to the longer after-ripening treatment. Only the most southerly source – Sacramento – did not benefit from the longest after-ripening treatment.

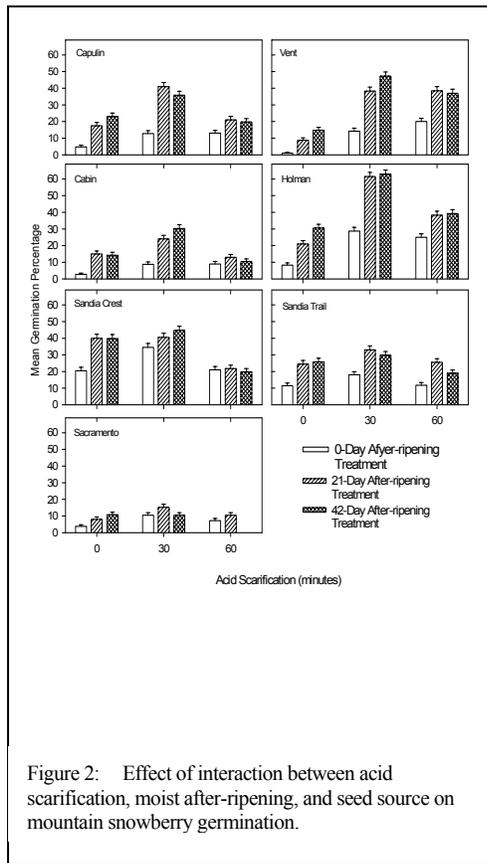


Figure 2: Effect of interaction between acid scarification, moist after-ripening, and seed source on mountain snowberry germination.

All snowberry seed sources benefited from some level of after-ripening treatment (when averaged across acid scarification treatments), but degree of germination improvement and optimal treatment level were variable (see Figure 1 (B)). A 21-day after-ripening treatment enhanced germination by 35% to over 160% depending on seed source. Latitude of seed source impacted response to after-ripening. Improvement in germination following either after-ripening treatment was greatest for the most northerly sources – Capulin, Vent, and Cabin.

Improvement was intermediate for Holman, the mid-latitude seed source. For the three most southerly seed sources – Sandia Crest, Sandia Trail, and Sacramento – after-ripening was less effective. For five seed sources (all except Vent and Sacramento) germination rates were similar following both after-ripening treatments. Only the Vent seed source had a higher germination rate when exposed to the longer after-ripening treatment. Only the most southerly source – Sacramento – did not benefit from the longest after-ripening treatment.

For all seed sources, the highest germination rates were seen with a combination of a 30-minute acid scarification treatment followed by either a 21-day or a 42-day after-ripening treatment (see Figure 2). For four of seven sources (Capulin, Cabin, Holman, and Sandia Crest) there was a marked decrease in germination when acid scarification duration was increased from 30 to 60 minutes in combination with either of the after-ripening treatments.

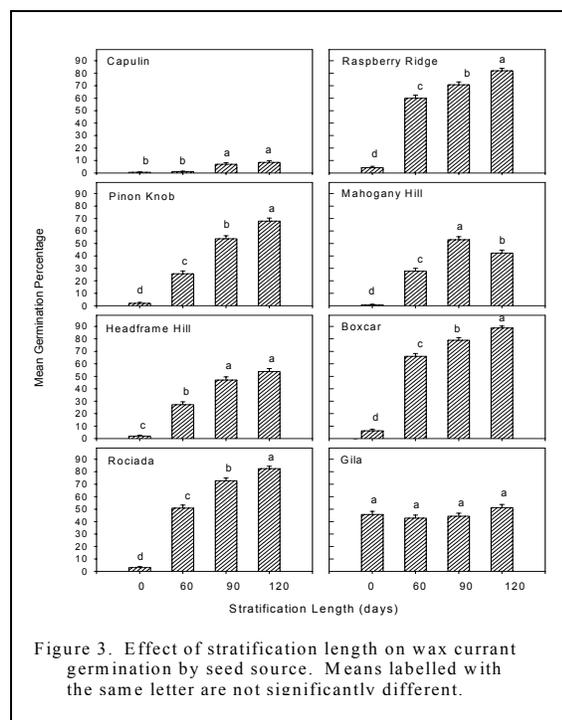
Stratification and its interaction with seed source impacted wax currant germination ($p < .05$). Increasing stratification length improved germination for seven of eight seed sources (see Figure 3). For those sources, this trend indicates variable stratification requirement within each seed lot. Stratification did not affect germination of the southernmost source (Gila). Averaged over all stratification treatments, germination by source was highly variable ranging from 4% to 60% (see Table 3). Looking at only the best stratification treatment for each source, germination ranged from 8.5% to 88.8%. There were no consistent differences in overall germination due to elevation or latitude of seed source.

Table 3 Wax currant percentages and standard errors for germination averaged across stratification treatments and by stratification treatment.

Seed Source							
Caplin	Raspberry Ridge	Pinon Knob	Mahogany Hill	Headframe Hill	Boxcar	Rociada	Gila

Table 3 Wax currant percentages and standard errors for germination averaged across stratification treatments and by stratification treatment.

	Seed Source							
	Caplin	Raspberry Ridge	Pinon Knob	Mahogany Hill	Headframe Hill	Boxcar	Rociada	Gila
Overall Germination	4.3 +/- 0.5	54.3 +/- 1.2	37.4 +/- 1.2	30.9 +/- 1.2	32.5 +/- 1.2	60.0 +/- 1.2	52.3 +/- 1.2	46.0 +/- 1.2
Stratification Control Germination	0.5 +/- 0.4	4.2 +/- 1.0	2.0 +/- 0.7	0.8 +/- 0.4	2.0 +/- 0.7	6.3 +/- 1.2	3.0 +/- 0.9	45.8 +/- 2.5
60-Day Stratification Germination	1.0 +/- 0.5	60.0 +/- 2.4	25.8 +/- 2.2	27.8 +/- 2.2	27.3 +/- 2.2	66.0 +/- 2.4	50.8 +/- 2.5	42.8 +/- 2.5
90-Day Stratification Germination	7.0 +/- 1.3	70.8 +/- 2.3	53.8 +/- 2.5	53.0 +/- 2.5	47.0 +/- 2.5	79.0 +/- 2.0	72.8 +/- 2.2	44.3 +/- 2.5
120-Day Stratification Germination	8.5 +/- 1.4	82.0 +/- 1.9	68.0 +/- 2.3	42.3 +/- 2.5	53.8 +/- 2.5	88.8 +/- 1.6	82.5 +/- 1.9	51.3 +/- 2.5



Discussion

Acid scarification and moist after-ripening treatments promote germination in the genus *Symphoricarpos* by degrading restrictive seed coats (Flemion and Parker 1942, Flemion 1934, Pfeiffer 1934). Evidence from studies on excised embryos indicates that seed coat-degrading treatments also affect the developing embryo, allowing subsequent maturation (Flemion 1934, Pfeiffer 1934). Moist after-ripening and acid scarification may alter chemical inhibitors in the seed coat allowing some developmental processes to occur during stratification that would otherwise be inhibited.

Previous work on common snowberry found that the combination of acid scarification and moist after-ripening was more effective than optimal level of either treatment alone (Flemion 1934, Pfeiffer 1934). This study found the combination of acid scarification and moist after-ripening to be best for all seed sources across a range of New Mexico latitudes and elevations.

Dormancy is an adaptive trait that prevents germination at times of year when a seedling would be unlikely to survive (Vleeshouwers et al. 1995). Common among temperate-zone shrub species, dormancy times germination to occur at the onset of the warm season (Baskin and Baskin 1998). For snowberry, however, a stratification requirement caused by embryo dormancy (Flemion 1934, Pfeiffer 1934, Flemion and Parker 1942) adequately prevents winter germination of the species. The scarification requirement for this species may serve other purposes.

Snowberry seeds are characterized by embryos that are initially immature and seed coats that are restrictive (Pfeiffer 1934). Both of these factors combine to ensure that embryos lack sufficient growth potential for germination until they have attained a high degree of maturation (Baskin and Baskin 1998). Maturation, which occurs during stratification, does not take place unless stratification is preceded by some seed coat-

degrading treatment (Pfeiffer 1934). This requirement delays germination to the second spring following dispersal or later. Variability in seed coat thickness likely results in some seeds requiring more than one warm season for adequate degradation to take place, thus spreading germination across time and ensuring the establishment of a seed bank.

Variability in depth of seed coat dormancy due to provenance may reflect adaptations to differing environmental conditions. Seed source variability in response to acid scarification has been found to occur in Kentucky coffeetree (*Gymnocladus dioicus*) (Ball and Kisor 1985). For that species, seeds collected in Minnesota did not benefit from acid scarification, while seeds collected in Ohio and Illinois did show a benefit. In this study, variability in scarification requirement was apparent in degree only. The three southernmost sources benefited less from acid scarification and after-ripening than did the four northernmost.

For wax currant, variability in the stratification requirement among seed sources is best explained by latitude of provenance. Germination increased with increasing duration of stratification for all northern New Mexico seed sources, while the southernmost source (Gila) germinated equally well with or without stratification. This result is consistent with the thought that for temperate woody species requiring stratification, seeds collected from sites with more severe winters would be expected to have a greater depth of dormancy than seeds collected from sites with milder winters (Meyer and Monsen 1991).

Wax currant seeds collected from northern New Mexico sites had highly variable stratification requirements within seed lots, consistent with a strategy of spreading germination over time and establishing a seedbank (Meyer and Kitchen 1994). Seeds from the southernmost source (Gila) lacked a stratification requirement. Lack of a stratification requirement indicates a lack of weather-predicting and seedbank-establishing mechanisms (Meyer and Kitchen 1994). However, stratified seeds from the Gila source germinated more rapidly than unstratified seeds. Slow germination has been shown to be an effective mechanism in preventing autumn germination of *Artemisia tridentata* (mountain big sagebrush) seeds (Meyer and Monsen 1991).

The emphasis on the use of local provenances for restoration is based on the premise that local ecotypes have adapted to local environments as an evolutionary response to particular selection pressures. Variability in seed propagation requirements among ecotypes suggests that seed dormancy characteristics are also adaptations to local environments in the face of the same selection pressures. The speed and completeness in which revegetation of a particular area can occur depends upon the ability of outplanted material to propagate itself on site. This characteristic of local plant material is as important as any other.

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